REPORT DOCUMENTATION PAGE

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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
03-07-2018	Final	Sept. 2014 - March 2018

4. TITLE AND SUBTITLE

Intravenous versus intramuscular cobinamide compared to intravenous saline (control) in the treatment of acute, survivable, hydrogen sulfide toxicity in swine (Sus Scrofa).

5a. CONTRACT NUMBER

5b. GRANT NUMBER

FWH20140070A

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

FWH20140070A

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

FWH20140070A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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10. SPONSOR/MONITOR'S ACRONYM(S)

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12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution A. Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Background: Hydrogen sulfide (H2S) is one of the leading causes of unintentional workplace gas inhalation deaths. Significant exposures (50-400 ppm) may produce difficulty in breathing, agitation, confusion, nausea and vomiting, elevated blood pressure and loss of consciousness. Several potential antidotes are available for hydrogen sulfide poisoning, but none have been completely successful. We previously reported that intravenous cobinamide successfully rescues severely NaHS-poisoned swine from apnea in the absence of assisted ventilation; however, intramuscular administration is preferred in a workplace or mass casualty scenario.

15. SUBJECT TERMS

cobinamide, hydroxocobalamin, nitrites, hydrogen sulfide, poisoning, toxicity, environmental, Vitamin B12a, Vit B12, pig, animal model, swine, sus scrofa

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Joseph K. Maddry
U	U	U	SAR		19b. TELEPHONE NUMBER *(Include area code)* 210-724-0654

59th Medical Wing
Institutional Animal Care and Use Committee (IACUC)
59 Clinical Research Division/SGVUS
1100 Wilford Hall Loop, Bldg 4430
Lackland AFB, TX 78236-5300

NOTICE OF ACTION REGARDING IACUC REVIEW

Date: 2 Mar 18

TO: Maj Joseph Maddry/SGO3D

Your **Final Report** was reviewed by the WHASC IACUC during the 13 Feb 18 meeting. The Committee's decision is provided below:

FWH20140070A, "Intravenous versus intramuscular // compared to intravenous saline (control) in the treatment of acute, survivable, hydrogen sulfide toxicity in swine (Sus Scrofa)." **PI: Maj Joseph Maddry/59 EMDS/SGO3D**

The committee voted that this item be **approved** as written. **FOLLOW-UP: CLOSED**

Name of Official	**Title/Office Symbol/Phone**
MARIA E. DOMINGUEZ	Office of Research Protocol Support /SGVUS 292-6095
Signature	Info Copy To

WHASC – Animal Final Report

09November2017

1. Protocol Number: FWH20140070A

2. Type of Research: Animal Research

3. Title: Intravenous versus intramuscular cobinamide compared to intravenous saline (control) in the treatment of acute, survivable, hydrogen sulfide toxicity in swine (Sus Scrofa).

4. Principal Investigator (PI):

Name	Rank	Date of IACUC Training	Branch of Service/ Corps	Staff Resident Fellow Civilian	Department / Office Symbol	Email (if other than WHASC Outlook)	Phone	Pager
Joseph Maddry	O-4	Jul 2015	USAF	Staff	59th EMDS	Joseph.maddry@gmail.com	210-916-3693	CP 210-630-7374

5. Purpose: This novel, reproducible model of hydrogen sulfide toxicity was developed to test drug treatments and/or resuscitation treatments that reverse the toxicity. To date there are no reproducible models of hydrogen sulfide toxicity although hydrogen sulfide toxicity is reported to be one of the leading causes of unintentional workplace gas inhalation deaths. Newer methods of extracting oil and gas such as hydraulic fracturing produce excessive levels of hydrogen sulfide gas. Hydrogen sulfide could also be used as a terrorist weapon in confined areas. There are no published reports indicating that a reproducible, lethal but survivable model of hydrogen sulfide toxicity have been developed. This model provides the ability to test any number of antidotes, drugs, or resuscitative measures in the treatment of hydrogen sulfide toxicity.

6. Results:
At the beginning of the study we had an unanticipated set back with the IM Cobinamide arm; we had difficulty resuscitating them despite varying the dose and timing of the Cobinamide. We shifted our focus to the IV arm. The NaHS was infused until the animal reached apnea, then titrated down twice and remained on 0.1mg/kg/min for the remainder of the study. Animals were treated with IV HOC, IV Cobinamide or control (no treatment) 1 minute post apnea. There were no significant differences in baseline variables. All of the IV Cobinamide treated animals survived, compared to IV HOC and control. Mean time to spontaneous ventilation in the Cobinamide treated animals was 3.2 minutes.

After completion of our IV arm we moved back to the IM model. We collaborated with Dr. Bebarta, under CRADA 16-206-AFMS59-C16004, at the University of Colorado, Denver, to complete the IM portion of the protocol. There were no significant differences in baseline hemodynamics and arterial blood gases were normal in both groups. There were no significant differences in the mg/kg dose of NaHS required to produce apnea or MAP less than 45 mmHg (9.04±6.16 mg/kg cobinamide vs. 5.90±5.54mg/kg control; 95% CI of difference 11.32, -5.04). All of the cobinamide treated animals survived (5/5), none of the control (0/6) animals survived. **Kaplan-Meier method of survival analysis clearly showed a significant difference by group (log rank p<0.001) such that more of the cobinamide treated animals survived compared to the untreated animals**. Mean systolic blood pressure 10 minutes post treatment was 101.20±17.06 mmHg for cobinamide treated animals vs 37.75±19.08 mmHg for control; 95% CI difference 2.94, 123.96.

7. How may your findings benefit the Air Force? Hydrogen sulfide is an attractive terrorism tool because of its high toxicity and ease with which it can be produced. Several potential antidotes have been proposed for hydrogen sulfide poisoning, but none have been completely successful. Our studies indicate that cobinamide can successfully reverse hydrogen sulfide poisoning in an animal model. We have also shown that cobinamide successfully reverses cyanide poisoning in an animal model. Moreover, cobinamide can be administered via the IM route making it an easy drug to deliver by first responders at any role of care.

8. Number of Animals

Projected Enrollment of Animals at the Beginning of Study: 62 (added 16 through amendments 1&4)

Actual Number of Animals Enrolled: 76

9. Status of Animals Entered into the Protocol: All animals used were in good health.

10. Number of animals since last status report:

	Number enrolled since last report	**Total enrollment to date**
Number of animals entered into the Study	0	76

11. Status of Funds: Our study was funded by the AF SGR. We had no budget deviations and all funds have been allocated for.

12. Reason for Closure: Objectives of the study were met.

13. Specific Problems: We completed 6 experiment animals treated with IM cobinamide and we had difficulty with resuscitating these animals. We expected that since the IV cobinamide worked so well, that the IM cobinamide would work almost as well. However, we were unsuccessful resuscitating these IM cobinamide treated animals despite varying the dose of cobinamide and timing of cobinamide administration. Because of this difficulty we obtained an amendment (#1) asking for 10 additional animals to further explore the IM dose of cobinamide to determine if it is being absorbed under these experimental conditions. With our initial IM model, 1/3 of the pigs survived. We worked toward improving the cobinamide formulation, which improved survival rate to 100%.

14. Publications and Presentations:

Presentations

2016 Oral presentation and poster at San Antonio Military Health System and Universities Research Forum (SURF) meeting in May 2016 at the University of Texas at San Antonio.

2017 Oral presentation and poster at the North American Congress of Clinical Toxicology meeting on October 11-15, 2017 in Vancouver, British Columbia at the Sheraton Vancouver Wall Centre.

These Presentations have been cleared by 59 CRD and Public Affairs.

Publications:
Bebarta VS, Garrett N, Brenner M, Mahon SB, Maddry JK, Boudreau S, Castaneda M, Boss GR. Efficacy of Intravenous Cobinamide Versus Hydroxocobalamin or Saline for Treatment of Severe Hydrogen Sulfide Toxicity in a Swine (Sus scrofa) Model. Academy of Emergency Medicine 2017 Sep;24(9):1088-1098. doi: 10.1111/acem.13213 [Electronic publication]

This publication has been cleared by the 59 CRD and Public Affairs

15. Exceptional Achievements: None

16. Signature of Principal Investigator:

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Director, USAF En route Care Research Center
Director, Clinical Resuscitation, Emergency Sciences, and Toxicology
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